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林下参内生球毛壳菌 FS-01 对人参病原真菌的抑制作用¹

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摘要: 为了研究林下参内生真菌球毛壳菌(Chaetomium globosum) FS-01 菌株对人参病原菌的抑菌作用,在实验室条件下,测定了 FS-01 菌株菌丝、发酵液和孢子悬浮液对人参黑斑病菌(Alternaria panax)、人参菌核病菌(Sclerotinia schinseng)、人参灰霉病菌(Botrytis cinerea)、人参立枯病菌(Rhizoctonia solani)、人参根腐病菌(Fusarium solani)5种人参病原菌的抑制作用。结果表明:内生真菌球毛壳菌 FS-01对 5种病原菌均有抑制作用,其中,对人参黑斑病菌的抑制作用最高,为 30.80%,其次是人参立枯病菌,人参菌核病菌,人参根腐病菌和人参灰霉病菌;发酵液抑菌实验结果表明,在加入内生真菌球毛壳菌 FS-01菌株发酵液的 PDA培养基上,对人参灰霉病菌的抑制作用最高,为 82.09%,其次是人参菌核病菌,人参黑斑病菌,人参立枯病菌和人参根腐病菌;孢子抑菌实验结果表明,在加入内生真菌球毛壳菌 FS-01菌株孢子悬浮液的 PDA培养基上,对人参黑斑病菌的抑制作用最高,为 83.72%,其次是人参灰霉病菌,人参立枯病菌,人参菌核病菌和人参根腐病菌。内生真菌球毛壳菌 FS-01菌株对人参病原菌均有很高的抑菌作用,可作为人参病原菌的生防菌株资源。

关键词: 林下参,内生真菌,球毛壳菌,人参病原菌,抑制作用

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Inhibitory effect of *Chaetomium globosum* FS-01against

ginseng pathogens

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Abstract: To study the inhibitory effects of Chaetomium globosum strain FS-01 against Alternaria panax, Sclerotinia schinseng, Botrytis cinerea, Rhizoctonia solani and Fusarium solani of ginseng pathogens, mycelia, fermentation broth and conidia suspension were tested in laboratory. The results showed that Chaetomium globosum FS-01 had a certain inhibition against the ginseng pathogens by confront culture. Among them, the highest inhibition rate to mycelia growth of Alternaria panax, reached 30.80%, followed by Rhizoctonia solani, Sclerotinia schinseng, Fusarium solani and Botrytis cinerea; On the PDA plate mixed with Chaetomium globosum FS-01 fermentation broth, the highest inhibition rate to mycelia growth of Botrytis cinerea, reached 82.09%, followed by Sclerotinia schinseng, Alternaria panax, Rhizoctonia solani and Fusarium solani; On the PDA plate mixed with Chaetomium globosum FS-01 conidia

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suspension, the highest inhibition rate to mycelia growth of *Alternaria panax*, reached 83.72%, followed by *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia schinseng* and *Fusarium solani*. Endophytic fungi FS-01 strain had a significant inhibitory effects, it could be utilized as a biological control resource inhibiting ginseng pathogens.

Key words: mountain-cultivated ginseng, endophytic fungi, *Chaetomium globosum*, ginseng pathogen, inhibitory effects

人参(Panax ginseng Meyer)属五加科(Araliaceae)人参属(Panax)多年生草本植物。这种植物具有巨大的药用价值,其根部常被用来增强脾肺功能,以及预防心血管疾病、脑血管疾病、慢性代谢综合症和糖尿病等(Briskin,2000; Shibata,2001; Yuan et al.,2012)。人参是一种在同一个地方生长缓慢的宿根植物。因此,人参面临着一系列的环境胁迫,包括细菌、真菌、线虫对其侵染。目前,国内外已有记载的人参病害有 40 多种,我国已发现的人参病害至少 25 种以上(王春伟,2011),严重影响了人参产业的健康发展。人参主要的病原菌有人参黑斑病菌(Alternaria panax)、人参菌核病菌(Sclerotinia schinseng)、人参灰霉病菌(Botrytis cinerea)、人参立枯病菌(Rhizoctonia solani)、人参根腐病菌(Fusarium solani)等(钟嘉丽等,2015; 张雷鸣等,2016; 卢占慧等,2016; 孙卓和杨利民,2015, 2016),直接影响了人参的产量和品质。

目前,防治人参病害主要利用化学药剂。化学药剂不仅价格昂贵而且对于防治病害的效果较差。此外,过度使用化学药剂会导致土壤中微生物环境的变化。况且,这些化学药剂对人类和植物有害,并且可能导致病原菌产生耐药性(Kalia & Gosal, 2011)。用植物内生菌防治人参病害国内外已有很多报道(Eo et al., 2014;李鹏祥等,2013)。内生菌被定义为其生活史全部或部分生活在植物组织内,通常对寄主植物不造成影响和伤害的微生物类群

(Rodriguez et al., 2009; Park et al., 2017)。内生菌在植物病害防治中扮演着重要的角色 (Chowdhury, 2017)。内生菌通过激活细胞对病原菌的防御反应,如氧化反应,加固细胞壁,相关防御酶反应和次生代谢产物积累等,从而增强宿主抵御病害的能力 (Ernst, 2010)。内生菌在植物体内具有稳定的生存空间,不易受环境条件的影响,因此,利用内生菌防治植物病原菌既是一种环境友好型又是一种经济有效的方法。

在本研究中,利用前期从林下参叶片分离的内生真菌球毛壳菌(Chaetomium globosum)为研究对象,明确球毛壳菌、孢子悬浮液和发酵液对人参病原菌的抑制作用,以期更好地防治人参主要病害,并为寻找新型生物菌肥的研制奠定基础。

1 材料与方法

1.1 材料

1.1.1 供试菌株

球毛壳菌菌株 FS-01 由中国农业科学院特产研究所实验室提供(周春元等, 2019)。

人参黑斑病菌(Alternaria panax)、人参菌核病菌(Sclerotinia schinseng)、人参灰霉病菌(Botrytis cinerea)、人参立枯病菌(Rhizoctonia solani)、人参根腐病菌(Fusarium solani)由吉林农业大学植物保护学院植物病理教研室提供。

1.1.2 供试培养基

PDA 培养基: 马铃薯 200 g, 葡萄糖 20 g, 琼脂 20 g, 蒸馏水 1 000 mL。

PD 液体培养基: 马铃薯 200 g, 葡萄糖 20 g, 蒸馏水 1 000 mL。

1.1.3 仪器与设备

HZQ-F160 振荡培养箱,哈尔滨市东联生化仪器有限公司; PL4002 精密电子天平,梅特勒-托利多仪器有限公司; BMJ 霉菌培养箱,上海博迅实业有限公司医疗设备厂; SW-CJ-IFD 洁净工作台,苏净集团苏州安泰空气技术有限公司; LDZX-30KBS 立式压力蒸汽灭菌器,

上海申安医疗器械厂; Nikon TS 倒置相差显微镜,日本尼康公司; PL4002 电子天平,梅特勒-托利多仪器上海有限公司。

1.2 方法

1.2.1 球毛壳菌 FS-01 与人参病原菌平板对峙培养

将保存的球毛壳菌 FS-01 菌株和供试人参病原菌菌株分别转接到 PDA 平板上,于 25 ℃ 黑暗条件下恒温培养 7 d 后,用 5 mm 打孔器在菌落边缘打取菌饼。采用两点对峙平板培养法,将人参病原菌菌饼分别接种于 PDA 平板一侧,距离人参病原菌菌饼 1 cm 处接入球毛壳菌菌饼,同时以只接种人参病原菌的平板为对照(CK),每个处理重复 3 次,置于 25 ℃黑暗条件下恒温培养 7 d,观察记录结果并拍照(刘彩云等,2015),按照下列公式计算抑制率:

$$T = (Rc - Rp) / Rc \times 100\%$$

式中: T 为抑菌率,Rc 为只接种人参病原菌菌落生长直径平均值,Rp 为对峙培养人参病原菌菌落生长直径平均值。

1.2.2 球毛壳菌 FS-01 发酵液对人参病原菌的抑制作用

1.2.2.1 发酵液的制备

将保存的球毛壳菌 FS-01 菌株在 PDA 平板上活化 7 d 后,在无菌的条件下,用 5 mm 打 孔器打取 10 个菌饼,转接到装有 100 mL PD 液体培养基的 250 mL 锥形瓶中,于黑暗条件下 25 $^{\circ}$ C、120 r • min⁻¹ 转速恒温摇床上振荡培养 7 d,无菌条件下,将培养的菌液经灭菌的 双层纱布过滤除去菌丝,滤液经 5 000 r • min⁻¹ 离心 15 min 去除沉淀,上清液经孔径为 0.22 m 的细菌过滤器过滤得到球毛壳菌 FS-01 无菌发酵液,置于冰箱(4 $^{\circ}$ C) 中保存备用(宋勇等,2018)。

1.2.2.2 发酵液对人参病原菌菌丝生长的抑制作用

吸取 2 mL 制备好的发酵液,加入 40~45 ℃的 PDA 平板中,混匀,以加入 2 mL 无菌水的平板作对照,将人参病原菌菌饼接种于平板中央,置于 25 ℃恒温培养箱中培养,培养7 d 后采用十字交叉法测量菌落生长直径 (方中达,1998),按照下列公式计算抑制率:

$$T = (Rc - Rp) / Rc \times 100\%$$

式中: T 为抑菌率,Rc 为无菌水处理平板中人参病原菌菌落生长直径平均值,Rp 为发酵液处理平板中人参病原菌菌落生长直径平均值。

1.2.3 球毛壳菌 FS-01 孢子悬浮液对人参病原菌的抑制作用

将球毛壳菌菌株在恒温培养箱中培养 7 d,用灭菌针刮取分生孢子,加入无菌水配置成孢子悬浮液,用血球计数的方法将溶液配成浓度为每毫升 1×10^7 个孢子悬浮液母液,随后将母液稀释成 1×10^6 、液稀释成 1×10^5 、液稀释成 1×10^4 、液稀释成每毫升 1×10^3 个不同浓度的孢子悬浮液。吸取 1 mL 制备好的悬浮液,加入 $40\sim45$ \mathbb{C} 的 PDA 平板中,混匀,以加入 1 mL 无菌水的平板作对照,将人参病原菌菌饼接种于平板中央,置于 25 \mathbb{C} 恒温培养箱中培养,培养 7 d 后采用十字交叉法测量菌落生长直径(索相敏等,2018),按照下列公式计算抑制率:

$$T = (Rc - Rp) / Rc \times 100\%$$

式中: $_T$ 为抑菌率, $_{Rc}$ 为无菌水处理平板中人参病原菌菌落生长直径平均值, $_{Rp}$ 为孢子悬液处理平板中人参病原菌菌落生长直径平均值。

2 结果与分析

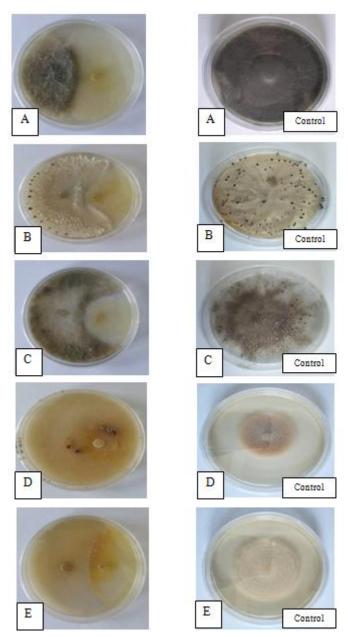
2.1 球毛壳菌 FS-01 菌丝对人参病原菌的抑制作用

由表 1 可以看出,球毛壳菌 FS-01 菌丝对人参病原菌均有不同程度的抑制作用,其中,对人参黑斑病菌的抑制作用最高,为 30.80%,其次是人参立枯病菌,人参菌核病菌,人参根腐病菌,抑制率分别为 24.37%、21.39%和 19.07%,对人参灰霉病菌抑制率最小,为 18.53%。当球毛壳菌 FS-01 菌丝与人参病原菌菌丝对峙培养 7 d 时,均会产生抑制区域,球毛壳菌 FS-01 菌丝与人参菌核病菌和人参灰霉病菌培养,产生的抑制区域明显,与人参黑斑病菌、人参立枯病菌和人参根腐病菌培养,产生的抑制区域不太明显(图 1)。

表 1 球毛壳菌 FS-01 菌丝对人参病原菌生长的抑制作用

Table 1 Inhibitory effect of Chaetomium globosum FS-01 against growth on ginseng pathogen

人参病原菌	生长直径	抑制率
Ginseng pathogen	Growth diameter (mm)	T (%)
人参黑斑病菌 Alternaria panax	42.11 ±2.58	30.80
CK	60.94 ± 5.36	
人参菌核病菌 Sclerotinia schinseng	43.52±3.67	21.39
CK	55.36±7.35	
人参灰霉病菌 Botrytis cinerea	45.23 ±2.57	18.53
CK	55.52±2.68	
人参立枯病菌 Rhizoctonia solani	37.18±1.59	24.37
CK	49.16±2.61	
人参根腐病菌 Fusarium solani	46.38 ± 2.56	19.07
CK	57.31 ±2.78	



注: A. 球毛壳菌 FS-01 与人参黑斑病菌对峙培养; B. 球毛壳菌 FS-01 与人参菌核病菌对峙培养; C. 球毛壳菌 FS-01 与人参灰霉病菌对峙培养; D. 球毛壳菌 FS-01 与人参立枯病菌对峙培养; E. 球毛壳菌 FS-01 与人参根腐病菌对峙培养。

Note: **A**. Confrontation culture of *Chaetomium globosum* FS-01 and *Alternaria panax*; **B**. Confrontation culture of *Chaetomium globosum* FS-01 and *Sclerotinia schinseng*; **C**. Confrontation culture of *Chaetomium globosum* FS-01 and *Botrytis cinerea*; **D**. Confrontation culture of *Chaetomium globosum* FS-01 and *Rhizoctonia solani*; **E**.

Confrontation culture of Chaetomium globosum FS-01 and Fusarium solani.

图 1 球毛壳菌 FS-01 与人参病原菌对峙培养

Fig.1 Confront culture of Chaetomium globosum FS-01 and ginseng pathogen

2.2 球毛壳菌 FS-01 发酵液对人参病原菌的抑制作用

由表2可以看出,球毛壳菌FS-01发酵液对人参病原菌均有不同程度的抑制作用,其中,对人参灰霉病菌的抑制作用最高,为82.09%,其次是人参菌核病菌,人参黑斑病菌和人参立枯病菌,抑制率分别为76.83%,74.04%和73.88%,对人参根腐病菌抑制率最小,为69.22%。

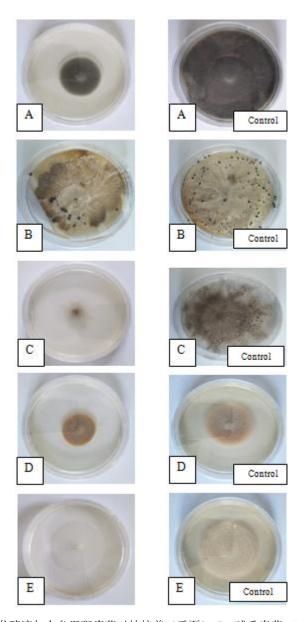
在球毛壳菌 FS-01 发酵液平板上,人参病原菌菌落被包围(图 2)。

表 2 球毛壳菌 FS-01 发酵液对人参病原菌生长的抑制作用

 ${\it Table 2 Inhibitory effect of \it Chaetomium \it globosum FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it Globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it Globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it Globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it Globosum \it FS-01 fermentation broth against growth on \it Chaetomium \it Globosum \it Globo$

ginseng pathogen

人参病原菌	生长直径	抑制率
Ginseng pathogen	Growth diameter (mm)	T (%)
人参黑斑病菌 Alternaria panax	16.14±2.37	74.04
CK	62.18±3.16	
人参菌核病菌 Sclerotinia schinseng	13.29±6.32	76.83
CK	57.36±5.19	
人参灰霉病菌 Botrytis cinerea	10.12 ± 2.18	82.09
CK	56.52±3.21	
人参立枯病菌 Rhizoctonia solani	12.58±2.75	73.88
CK	48.16±3.41	
人参根腐病菌 Fusarium solani	17.27 ±2.17	69.22
CK	56.11 ±3.46	



注: A. 球毛壳菌 FS-01 发酵液与人参黑斑病菌对峙培养(反面); B. 球毛壳菌 FS-01 发酵液与人参菌核病菌对峙培养(反面); C. 球毛壳菌 FS-01 发酵液与人参灰霉病菌对峙培养(反面); D. 球毛壳菌 FS-01 发酵液与人参立枯病菌对峙培养(反面); E. 球毛壳菌 FS-01 发酵液与人参根腐病菌对峙培养(反面)。
Note: A. Confrontation culture of Chaetomium globosum FS-01 fermentation broth and Alternaria panax (reverse side); B. Confrontation culture of Chaetomium globosum FS-01 fermentation broth and Sclerotinia schinseng (reverse side); C. Confrontation culture of Chaetomium globosum FS-01 fermentation broth and Botrytis cinerea (reverse side); D. Confrontation culture of Chaetomium globosum FS-01 fermentation broth and Rhizoctonia solani (reverse side); E. Confrontation culture of Chaetomium globosum FS-01 fermentation broth and Fusarium solani (reverse side).

图 2 球毛壳菌 FS-01 发酵液对人参病原菌的抑制作用 Fig.2 Inhibitory effects of *Chaetomium globosum* FS-01 fermengtation broth against ginseng pathogen

2.3 球毛壳菌 FS-01 孢子悬浮液对人参病原菌的抑制作用

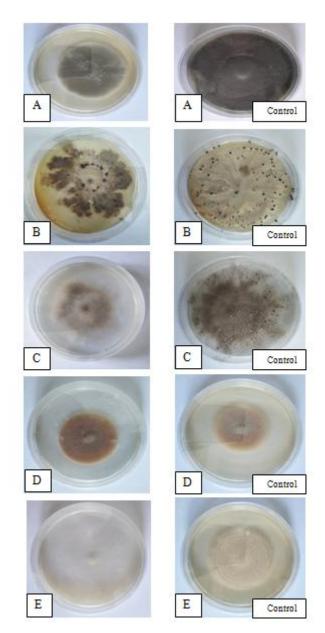
当孢子悬浮液浓度为每毫升 1×10⁷ 个时, 球毛壳菌 FS-01 孢子悬浮液对人参病原菌均有

不同程度的抑制作用,其中,对人参黑斑病菌的抑制作用最高,为83.72%,其次是人参灰霉病菌,人参立枯病菌,人参菌核病菌,抑制率分别为81.99%,76.19%和68.77%,对人参根腐病菌抑制率最小,为62.19%(表3)。在球毛壳菌FS-01孢子悬浮液平板上,人参病原菌菌落被包围(图3)。

表 3 球毛壳菌 FS-01 孢子悬浮液对人参病原菌生长的抑制作用

Table 3 Inhibitory effects of Chaetomium globosum FS-01 conidia suspension against growth on ginseng

pathogen				
人参病原菌	生长直径	抑制率		
Ginseng pathogen	Growth diameter (mm)	$T\left(\% ight)$		
人参黑斑病菌 Alternaria panax	10.12±3.26	83.72		
CK	62.18±3.16			
人参菌核病菌 Sclerotinia schinseng	18.15 ±2.18	68.77		
CK	58.12±3.58			
人参灰霉病菌 Botrytis cinerea	10.32 ± 1.27	81.99		
CK	57.31 ±1.37			
人参立枯病菌 Rhizoctonia solani	11.23 ±2.69	76.19		
CK	47.16±7.52			
人参根腐病菌 Fusarium solani	21.28±3.52	62.91		
CK	57.36±1.29			



注: **A.** 球毛壳菌 FS-01 孢子悬浮液与人参黑斑病菌对峙培养(反面); **B.** 球毛壳菌 FS-01 孢子悬浮液与人参菌核病菌对峙培养(反面); **C.** 球毛壳菌 FS-01 孢子悬浮液与人参灰霉病菌对峙培养(反面); **D.** 球毛壳菌 FS-01 孢子悬浮液与人参立枯病菌对峙培养(反面); **E.** 球毛壳菌 FS-01 孢子悬浮液与人参根腐病菌 对峙培养(反面)。

Note: **A.** Confrontation culture of *Chaetomium globosum* FS-01 spore suspension and *Alternaria panax* (reverse side); **B.** Confrontation culture of *Chaetomium globosum* FS-01 spore suspension and *Sclerotinia schinseng* (reverse side); **C.** Confrontation culture of *Chaetomium globosum* FS-01 spore suspension and *Botrytis cinerea* (reverse side); **D.** Confrontation culture of *Chaetomium globosum* FS-01 spore suspension and *Rhizoctonia solani* (reverse side); **E.** Confrontation culture of *Chaetomium globosum* FS-01 spore suspension and *Fusarium solani* (reverse side).

图 3 球毛壳菌 FS-01 孢子悬浮液对人参病原菌的抑制作用 Fig.3 Inhibitory effects of *Chaetomium globosum* FS-01 conidia suspension against ginseng pathogen

3 讨论

目前多采用五氯硝基苯、噁霉灵、多菌灵、咯菌腈、代森锰锌、多抗霉素、丙环唑、嘧菌酯等多种化学农药已被广泛用于防治人参病害,但研究表明人参病原菌对以上农药逐渐产生了抗药性(Saito et al., 2016),亟需寻求新的防治人参病害的方法,以减缓病原菌抗药性的产生。内生菌生存在植物体内,有稳定的生存空间,可以长期地定殖于植物体内,不易受外界环境条件的影响,与病原菌可以直接互作,是一类重要的生防菌资源(Aly et al.,2011)。

球毛壳菌(Chaetomium globosum)是毛壳菌中研究最早的生防菌(Martin & Moore, 1995),具有产生抑菌物质的能力(Soytong et al., 2001),广泛应用于病害的防治。印容等(2016)研究表明球毛壳菌(Chaetomium globosum)产生的鞘氨醇类物质,对油菜根肿菌具有较强的抑制作用,兰楠等(2011)从油菜中分离到的内生球毛壳菌 YY-11 在平板对峙试验中,对油菜菌核病菌(Sclerotinia sclerotiorum)、立枯丝核菌(Rhizoctonia solani)、棉花立枯菌(Rhizotonia solani)、棉花枯萎病菌(Fusarium oxysporum)、油菜白斑病菌(Ce rosphorella albo-maculans)、油菜黑斑病菌(Alternaria brassicae)、油菜灰霉病菌(Botrytis cinerea)、小麦赤霉菌(Fusarium graminearum)都有抑制作用。本研究以实验室前期获得的优势菌株球毛壳菌 FS-01 为材料,通过内生菌菌丝对峙培养,发酵液和孢子悬浮液抑菌试验,研究了其对 5 种人参病原菌的抑制作用。研究结果表明:球毛壳菌 FS-01 菌株对 5 种人参病原菌均有不同程度的抑制效果,具有广谱性,可作为一种潜在的人参病害生物农药。但是,内生真菌球毛壳菌 FS-01 在活体上对病原菌的抑制能力是否与体外一致,以及抑菌机制、菌株发酵液中抗菌物质的活性成分和化学结构还有待进一步深入研究。

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